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Influence of a 3-step disinfection procedure on dentin bond strength

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School of Dentistry—Graduate Prosthodontics
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This is to certify that the thesis prepared by Maria T. Pappas, D.M.D., entitled INLUENCE OF A 3-STEP DISINFECTION PROCEDURE ON DENTIN BOND STRENGTH has been approved by her committee as satisfactory completion of the thesis requirement for the degree of Master of Science.

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October 29, 2004

INFLUENCE OF A 3-STEP TOOTH DISINFECTION PROCEDURE ON DENTIN BOND STRENGTH

A thesis submitted in partial fulfillment of the requirements for the degree of Master of Science at Virginia Commonwealth University

By

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Abstract

INFLUENCE OF A 3-STEP TOOTH DISINFECTION PROCEDURE ON DENTIN BOND STRENGTH

By Maria T. Pappas, D.M.D.

A thesis submitted in partial fulfillment of the requirements for the degree of Master of Science at Virginia Commonwealth University.

Virginia Commonwealth University, 2005.

Major Director: Dr. David R. Burns
Professor and Program Director
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Statement of problem. Clinicians have used disinfection materials to remove surface contaminants during cavity preparation. It has been postulated that disinfection materials may negatively affect shear bond strength of restorative materials. If so, large numbers of restorations may be predestined for early failure with the use of a disinfection protocol.

Purpose. To determine whether there is a difference in the bond strength between dentin and composite resin with a 3-step disinfection technique compared to a conventional bonding technique without the additional disinfection protocol.

Material and Methods. Sixty human molar teeth were sectioned parallel to the occlusal surface to expose mid-coronal dentin and mounted parallel to a bond shearing device on a universal testing machine (Instron) and randomly divided into 2 groups. In Group I (n=30), specimens were treated with chlorhexidine, tubulicid red, and sodium hypochlorite before dentin bonding, following the manufacturer's instructions for All Bond 2. In Group II (n=30), specimens were treated only with the bonding protocol of All Bond 2. To ensure a uniform bond surface area, core paste was syringed into a cylindrical mold (diameter 2.38mm, height 2.00mm) that was in contact with the dentin bonding surface of each specimen, and allowed to set under constant force. All specimens were subjected to fracture by shear loading in a universal testing machine (Instron) at a uniform crosshead speed of 0.02 inch per minute and expressed as MPa. Statistical analysis, using nonpaired student's t-test, was performed.

Results. A statistically-significant higher shear bond strength was found for the 3-step disinfection group (mean shear bond strength, 25.3; STD, 4.6) compared to the conventional bonding group (mean shear bond strength, 20.5, STD, 3.4) ($P<.0001$).

Conclusions. The 3-step disinfection technique group showed a significantly stronger dentin shear bond strength compared to the conventional bonding technique without disinfection.

CHAPTER 1. Introduction

Cavity preparation involves surgical removal of infected dentin (caries) using a handpiece and/or hand instruments and the shaping of the cavity to prepare it to receive restorative materials. During cavity preparation, cutting debris is smeared over the enamel and dentinal surfaces, forming what is termed the "smear layer."[1] The smear layer consists of pulverized hydroxyapatite, altered collagen, saliva, bacteria, and other grinding surface debris.[2] This smear layer can have a negative influence on the adhesive bond formed between the tooth and restorative material.[3] Factors influencing smear layer removal and subsequent adhesive bonding include: treatments removing contaminating handpiece oil residue, saliva, and bacteria, dentinal wetness associated with etching procedure, and bonding agents used in the restorative procedure.[4]

Oil lubricants are required for the proper maintenance of handpieces. During cavity preparation, handpiece lubricant may be inadvertently left behind, creating "surface contamination."[5] This contaminated surface has been shown to negatively affect bond strength.[6] Teeth prepared with a lubricated handpiece were directly compared to teeth prepared with a dry handpiece.[6] Those teeth prepared with the dry handpiece had significantly higher bond strengths than teeth that were prepared with a lubricated handpiece. Introduction of saliva into the prepared site also has been shown to weaken bond strength of composite material to tooth structure.[7] Artificial saliva and human plasma lower the bond strengths to dentin by 100%, confirming that protein contamination decreases the bond strength of dentin bonding agents.[3, 7] Introduction of bacteria into the cavity preparation is a frequent occurrence. In fact, the histological and bacteriological experiments performed to determine whether viable organisms remain on the dentinal surface at the termination of routine cavity preparation have shown that only a proportion of the teeth are sterile after preparation.[8, 9] Crone[8] showed that half of the prepared teeth investigated by histological techniques still contained microorganisms. Besic[10] reported that bacteria left in a cavity preparation could survive for longer than 1 year. These bacteria left in the cavity preparation may lead to secondary caries under the restoration and create further destruction of the remaining tooth structure.[10]

Attempts have been made to remove surface contaminants during cavity preparation. Devices have been used to control saliva during dental treatment including rubber dam, cotton rolls, absorbent papers, saliva ejectors, and high volume evacuators. Commercially- available disinfectants containing compounds such as chlorhexidine digluconate, disodium EDTA dihydrate, sodium hypochlorite, hydrogen peroxide, and iodine have been used to remove oil and bacterial contaminants. Chlorhexidine contains chlorhexidine gluconate, which binds to the amino acids in the dentin and continues to kill bacteria for several hours,[11] making it a good antimicrobial agent.[12-21] Application of chlorhexidine before or after an acid-etching procedure has been shown to increase bond strength.[14] Tubulicid red was demonstrated to interfere with bacterial penetration in the unprotected dentinal tubules.[22-23] This compound contains disodium EDTA dihydrate and 1.0% sodium fluoride and is an effective smear layer remover. In a study by Surmont et al.,[22] the use of tubulicid red did not affect tensile bond strength. Sodium hypochlorite (NaOCl) is another antimicrobial agent that has been used traditionally as an irrigating solution in infected root canals. There have been several studies that investigated the role of sodium hypochlorite used as a disinfectant prior to a bonding procedure.[24-29] Van Dijken[24] demonstrated that acceptably-bonded restorations were achieved only after the pretreatment of a tooth with hypochlorite. Other studies[25-28] have also demonstrated that if acid-etching is followed by NaOCl treatment, higher bond strengths could be achieved. Saboia and others[28] comment on the positive effect of NaOCl on the bond strength of an acetone-based adhesive, due to the higher diffusibility of the acetone, as well as the higher capacity to displace water.

In addition to contamination, bonding strength is affected by dentinal wetting and the penetration of the dentinal surface by the bonding agent. Successful bonding requires two adhering surfaces to be in close proximity. This penetration can be influenced by external and internal dentinal wetness. External wetting of a surface by a liquid source is characterized by the contact angle of a droplet placed on the surface. This contact angle ranges from complete wetting of a surface, zero degrees, to various degrees of wetting. The greater degree of the contact angle, the weaker the bond strength.[2] The contact angle relates to the surface tension of the dentinal surface, the bonding agent (consisting of primer and resin), and the interface between the two. Surface energy of etched dentin is influenced by the amount of organic substance (predominantly collagen fibrils) in the substrate.[30] This surface energy can also be influenced by internal dentinal wetness. This wetness is a consequence of dentinal permeability, provided by the presence of dentinal tubules.[31] Dentinal tubules exude fluid that can interfere with the adhesive by blocking surface availability during bond formation. [31] However, moisture (water) is necessary as it keeps the hybrid layer of collagen expanded so the primer and resin monomers can penetrate the etched dentin surface to create a bond.

Improved adhesive bonding is also affected by the bonding agent used in the restorative procedure. The first and second-generation adhesives both had low bond strengths.[4,32-34] Subsequent third and fourth generation adhesives showed improvements in bond strength with fourth generation being superior due to its capacity for complete removal of the smear layer. The fourth-generation adhesives involve a technique of pre-treating the dentin with conditioners and/or primers that make the dentin more receptive to bonding. This technique also involves the application of an adhesive resin that copolymerizes with the primed dentinal surface and offers bonding receptors for copolymerization with the restorative material. In general, dentin bonding systems work by removing the smear layer, demineralizing the intertubular dentinal surface, and penetrating the surface to surround the exposed collagen fibrils, followed by polymerization. Hydrophilic bonding resin then forms a hybrid zone of polymerized resin and collagen dentin, creating a bond between the restorative material and dentin.[35]

High dentinal permeability allows bacteria and toxins to easily penetrate the dentinal tubules to the pulp, if the tubules are not sealed. [36] All the above mentioned disinfectants have been tested individually, or with other combinations to determine their effectiveness in reducing bacterial microleakage and their ability to affect the bonding strength of the restorative material.[12-28] Recently, however, there has been a new disinfection technique that involves the combination of chlorhexidine, tubulicid red, and sodium hypochlorite respectively, prior to a bonding procedure. This procedure may eliminate possible contaminants from the tooth surface prior to bonding a core buildup resulting in higher bond strength. The purpose of this paper is to determine whether there is a difference in the amount of bond strength between the use of a 3-step disinfection technique compared to a conventional bonding technique without the use of disinfection.

CHAPTER 2. Materials and Methods

Sixty intact, caries-free extracted human molars were stored in 6% NaOCl solution for 24 hours and then in tap water at room temperature. A low-speed diamond saw (Buehler, Lake Bluff, IL) with water coolant was used to section the teeth parallel to the occlusal plane to expose mid-coronal dentin. Any enamel flash remaining was smoothed with a carbide disc. One-inch diameter phenolic mounting cylinders (Buehler, Lake Bluff, IL) were filled with autopolymerizing tray resin (Dentsply International, York PA), allowed to polymerize completely, and then drilled with a 3/8 inch end mill to provide space for each tooth. Teeth were mounted inside the cylinders using light polymerizing acrylic resin (Triad, Dentsply International, York PA) placed in the drilled hole to secure each tooth and polymerized after determination that the bonding surface was parallel to the Instron bond shearing device. The parallel position between the tooth bond surface and the Instron bond shearing device was determined by lack of light detection between the shear bar and tooth surface when placed in contact while in the test device. Teeth were randomly divided into 2 groups (n=30). The specimens were ultrasonically cleaned with distilled water for 60 seconds to remove debris, and subsequently dried with a gentle stream of filtered air.

In group I, the dentinal surfaces were treated with 32% phosphoric acid solution (Uni-Etch with BAC, Bisco Dental Products, Scaumburg, IL) for 15 seconds and the teeth were then rinsed thoroughly, but not dried. Ten applications of Primer A & B (All Bond 2 Kit, Bisco Dental Products) were applied to the teeth from a clean disposable dappen dish (Dapaway, Crescent Dental Manufacturing, York, PA) with a bend-a-brush (Centrix, Shelton, CT). The teeth were thoroughly dried using light air pressure for at least 5 seconds with a totally clean & dry air system syringe (Best Buy Discount Dental Supply, Orlando, FL). Dentin Enamel Bonding Resin (All Bond 2 Kit, Bisco Dental Products) was applied from a disposable dappen dish (Dapaway) with a bend-a-brush (Centrix) and polymerized for 20 seconds. The specimens were placed under an Ultradent (Ultradent Products, South Jordan, Utah) bonding device in order to bond a uniform amount of composite material onto the dentin surface. The Ultradent bonding device contains a cylindrical mold that provides a standardized restorative material size and bond surface area with defined bond area of 2.38mm diameter and 2.00mm height. Room-temperature enamel-shade core paste (Den-Mat, Santa Maria CA) was auto-mixed and syringed using a needle tube with an S class syringe (Centrix) through the Ultradent bonding mold and onto the tooth surface. The bonding device was then secured to the tooth surface and excess core paste material was removed using a plastic instrument (Hu-Friedy, Chicago, IL).

In group II, the dentinal surfaces were scrubbed with a 4% chlorhexidine solution (Dial, Scottsdale, AZ) using a 3-cc syringe with an inspiral brush tip (Ultradent, South Jordan, UT) for 15 seconds. Next, the dentinal surfaces were scrubbed with tubulicid red (Global Dental Products, North Bellmore, NY) using a 3-cc syringe with a blue mini-dento infusor tip (Ultradent, South Jordan, UT) for 15 seconds. Lastly, the tooth surfaces were scrubbed thoroughly for 15 seconds with 6.0% NaOCl (Clorox, Oakland, CA) using a 3-cc syringe with an inspiral brush tip (Ultradent, South Jordan, UT) and the surfaces rinsed with distilled water and dried with filtered air. The All Bond 2 bonding protocol followed by the core paste was performed on each specimen using the identical protocol described above.

All the specimens were allowed to set under constant force of 5 pounds for 15 minutes in an incubator at mouth temperature (37^{o} C) using a polyvinylsiloxane (3M Express, 3M Dental Products, St. Paul, MN) putty mold placed over the mounting device and held in place by a weight (Figure 1). The specimens were removed from the pressure device and allowed to set in the incubator 37^{o}C for an additional 30 minutes with the polyvinylsiloxane mold still in place. The specimens were then carefully separated from the mold by lifting the Ultradent device while securing the sample with a plastic instrument (Hu-Friedy) to allow the bond to remain undisturbed.

All the specimens (n=60) were then stored in distilled water for 7 days in the incubator at 37^{o}C. Excess core paste material flash was removed with the aid of a microscope (30X) and 25 scalpel blade to standardize the bond area. Specimens were placed in the appropriate loading device and tested for shear bond strength using the crosshead pin mounted in a universal testing machine (Instron Corp., Canton, MA) at a crosshead speed of 0.02” per minute (Figure 2, Figure 3). The force required to fracture the specimen was recorded in pounds (lbs.) and the shear bond strength was calculated as the ratio of fracture load and bonding area and expressed in megapascals (MPa).

The mode of failure was noted after a visual examination using a light microscope under 30X magnification. Failures were recorded as adhesive (those occurring between the core material and tooth), cohesive (those occurring within the core material or tooth) or mixed (combination of adhesive and cohesive).

Figure 1. Specimen composite material polymerizing under pressure. Specimens were loaded with composite, sealed with a polyvinylsiloxane mold, and allowed to set under constant force of 5 lbs.

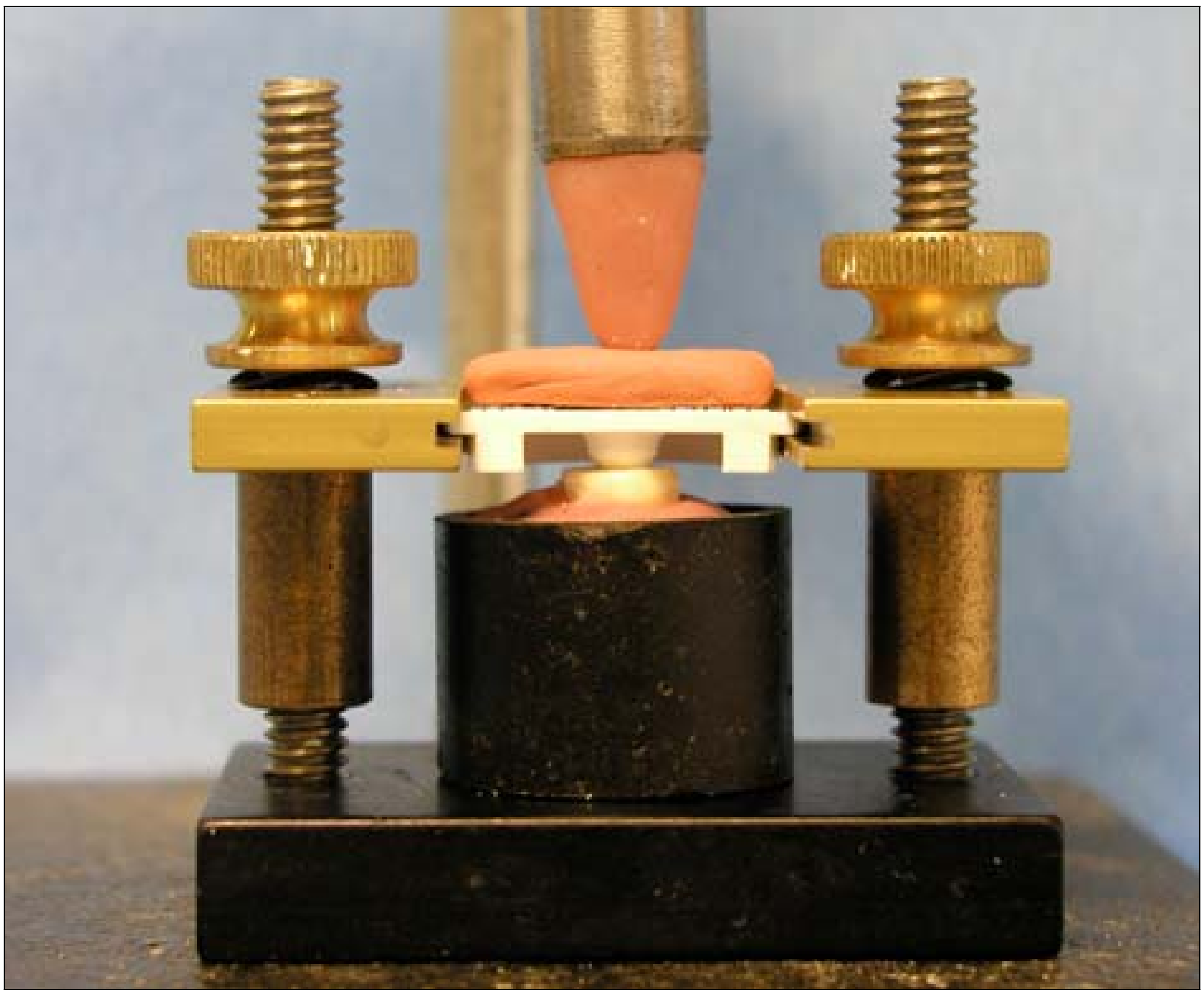


Figure 2. Frontal view of shear bond strength test. Specimens were placed into a device and mounted for shear test loading in a universal testing machine (Instron).

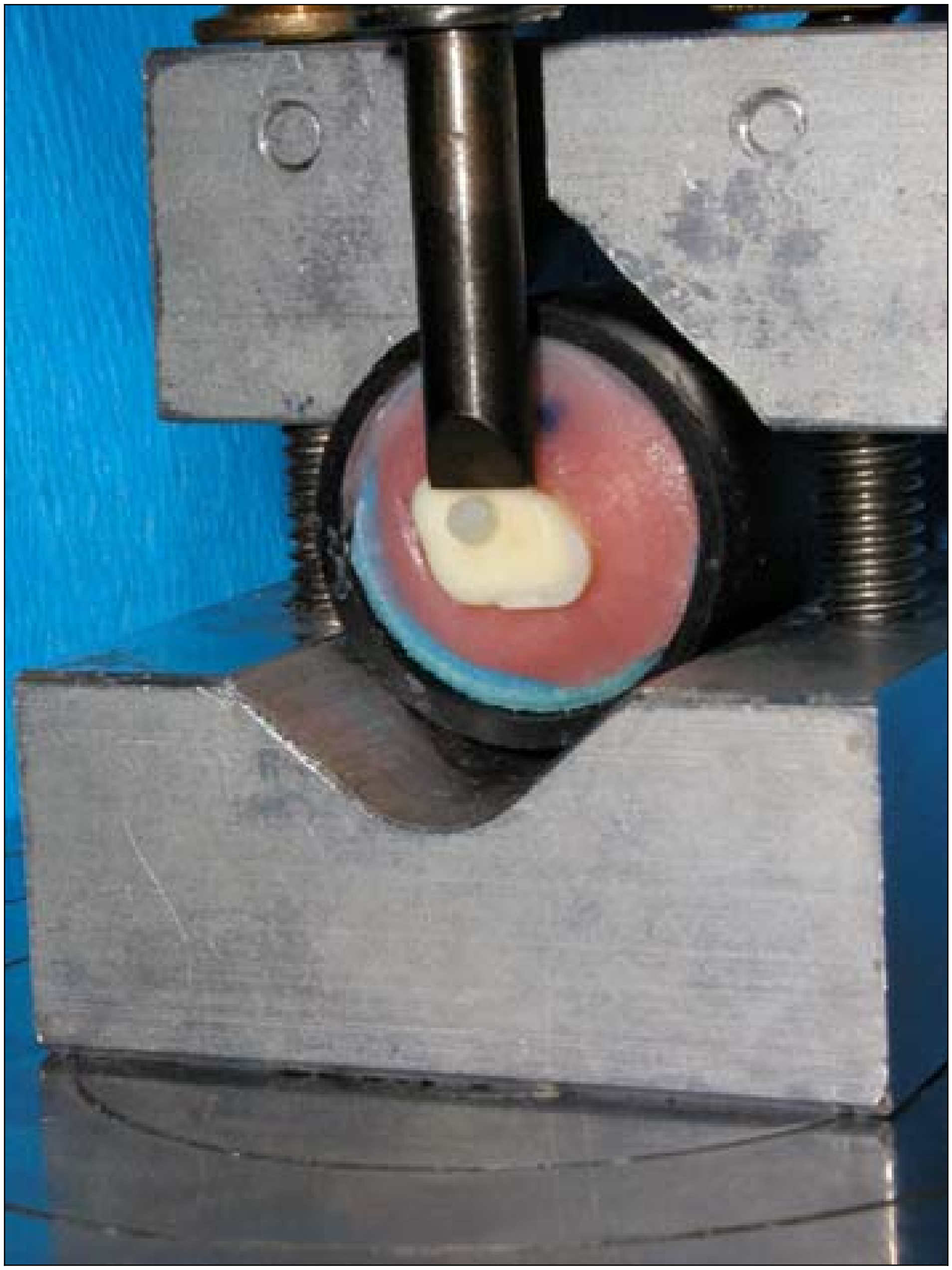
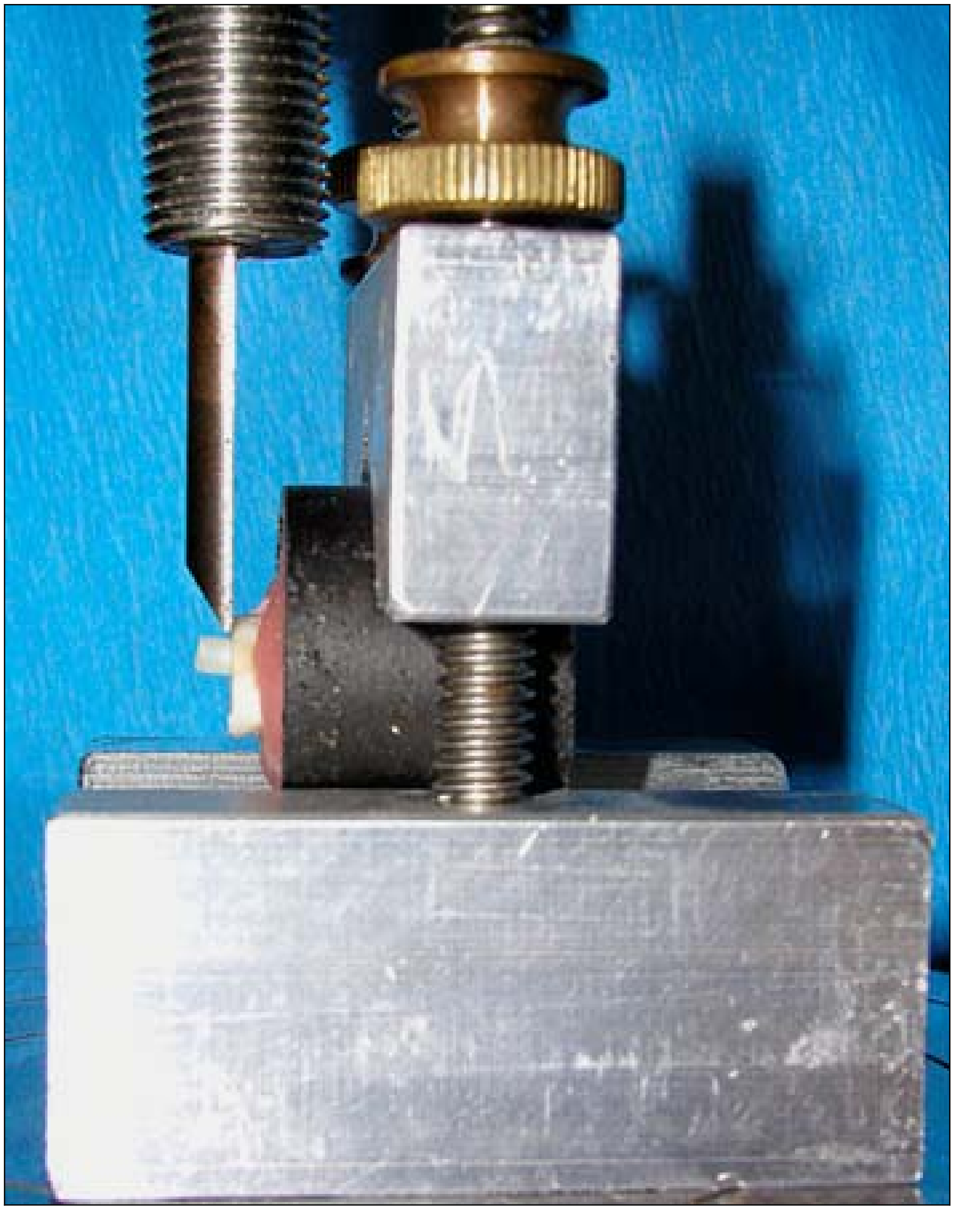


Figure 3. Side view of shear bond strength test. Specimens were placed into a device and mounted for shear test loading in a universal testing machine (Instron).



CHAPTER 3. Results

Using statistical analysis software (GraphPad, InStat Version 3.00 Windows 95, San Diego CA), Figure 3 and Table 1 were developed to show the distribution of bond strengths for both groups. The 3-step disinfection produced higher strengths (mean shear bond strength, 25.3; STD, 4.6) compared to the conventional bonding group (mean shear bond strength, 20.5, STD, 3.4). A student's *t* test comparison of the bond strengths demonstrated a statistically significant difference ($P<.0001$) between the two groups.

Table 2 reports the types of failure noted under visual examination at 30X magnification. Most failures were adhesive for the control group, but the fracture modes were more evenly divided for the 3-step disinfection protocol group. Figure 4 shows a graph of shear bond strength of the means of the types and numbers of failures that occurred within the two groups. The decreased number of weaker adhesive failures in the 3-step disinfection group suggests that the disinfection procedure improved the interfacial bond.

Table 1. Shear bond strength (MPa) of the 3-step disinfection and control groups

Groups	**n**	**Mean**	**Std. Dev.**	**Lower 95%**	**Upper 95%**
3-step disinfection protocol	30	25.3	4.6	23.6	26.9
Control	30	20.5	3.4	19.3	21.7

Figure 4. Plot of shear bond strength (MPa) for the 3-step disinfection and control groups. The mean is indicated by the center point and the standard deviation indicated by the outlying lines.

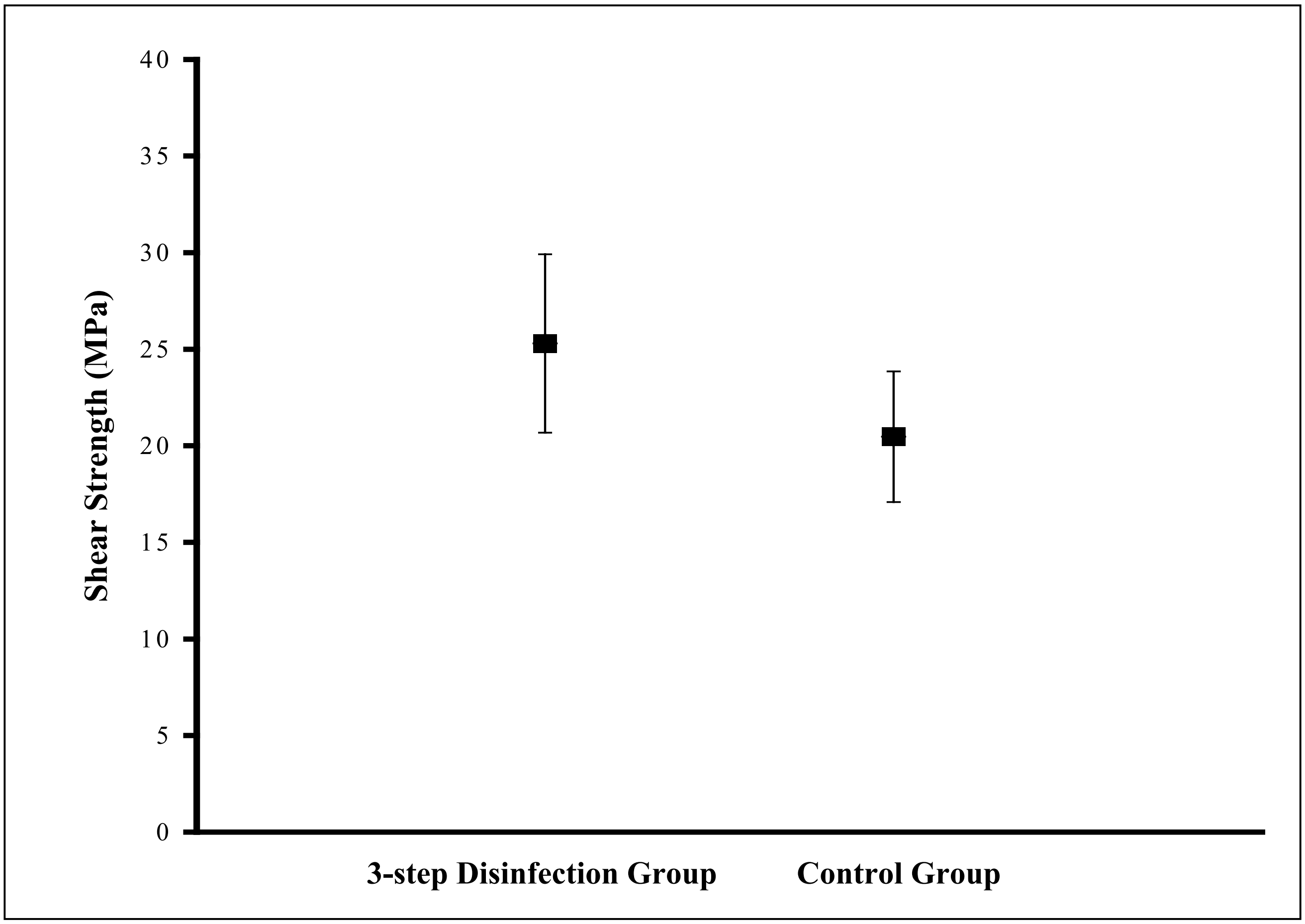
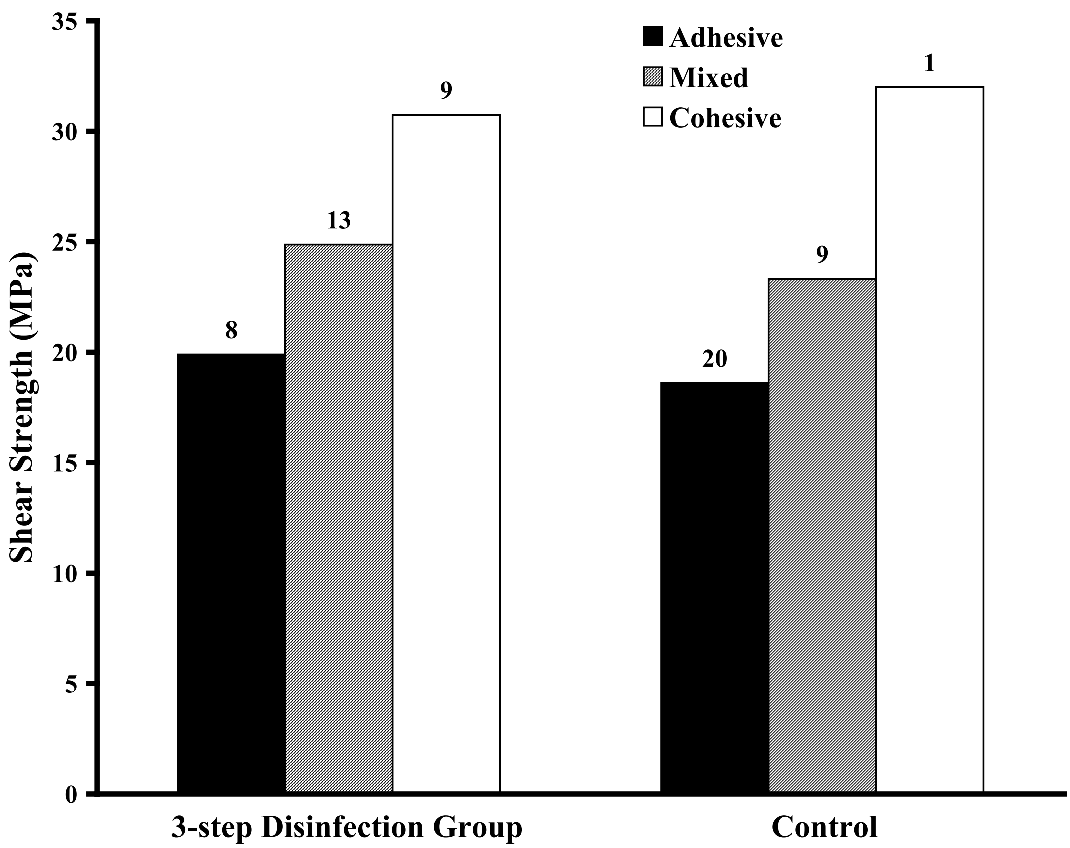


Table 2. Types of failure (adhesive, cohesive, or mixed) for the 3-step disinfection and control groups at 30X magnification as percent (%) of n.

Groups	n	Adhesive	Cohesive	Mixed
3-step disinfection group	30	26.7%	30.0%	43.3%
Control	30	66.7%	3.3%	33.0%

Figure 5. Graph of shear bond strength of the means in MPa of the types and numbers of failures that occurred within the two groups.



CHAPTER 4. Discussion

Shear bond strength tests a combination of tensile and compressive forces between a material and tooth surface, as well as within the material. When a restoration is bonded to a tooth, the bonded tooth surface is subjected to a combination of these forces. This study attempted to determine whether a 3-step disinfection protocol prior to a bonding procedure would result in higher bond strengths when compared to the control group without the disinfection protocol. Each of the three materials included in this protocol has been independently studied,[12-28] but never in conjunction and in this specific sequence.

In the present study, the specimens were initially treated with chlorhexidine, a material that binds to the amino acids in the dentin and continues to kill bacteria for several hours.[11] Studies have shown that the application of chlorhexidine [15-18, 21] before etching does not affect shear bond strength, in fact, application of chlorhexidine may increase the shear bond strength.[14]

The subsequent application of tubulicid red, also an antimicrobial agent, aids in removing the smear layer and leaving the smear plugs intact.[34] Its effects on shear bond strength may be considered minimal, due to lack of dentin permeability.[34] Sodium hypochlorite, the last antimicrobial agent used in this 3-step disinfection process may be the key factor in obtaining higher bond strengths. Sodium hypochlorite treatment can increase the diameter of tubule orifices,[27] allowing the bonding agent to penetrate 5-10mm below the smear layer.[34] This results in a greater surface area for adhesive penetration, which has been shown to be directly related to increased bond strength.[27] Thus, it is possible that the application of NaOCl may have increased bond strength independent of applying chlorhexidine and tubulicid red.

One of the other key factors that can influence bond strength is the type of dental substrate. Dentin has a heterogenous surface consisting of approximately 30% organic matter by volume, and subsequently has a lower surface energy.[2] Since the extracted teeth that were used in this study were not controlled for age, the dentin surface can vary from one tooth to the next as the dentinal tubule diameter varies with age. [4,31] Also, dentinal tubules change in size from the surface to the pulp chamber creating a variation in dentin bond strength within the same tooth, depending on the bond site.[31] This may be one of the factors contributing to the ranges in bond strength obtained.

All Bond 2, the adhesive material used in this study has an acetone solvent that removes the residual moisture, enhances resin wetting, and may counteract the adverse effects of organic contamination on bonding to tooth structure.[5] The interaction of NaOCl on this acetone-based adhesive may have allowed for increased diffusibility of the acetone, as well as higher capacity to displace water within the tubules.[28] These two factors may improve the contact of the monomer with the intertubular dentin substrate and promote higher bond strength.[28] This increase in bond strength in the disinfection group may explain the greater number of cohesive failures as compared to the control group, which had more adhesive failures. The specimens with more adhesive failures also had lower bond strengths, indicating a weaker resin-dentin bond.

The three antimicrobial agents used in this study are currently used to remove saliva, oil, and bacteria left on the tooth surface after cavity preparation. Further research is needed to independently assess the individual effects of each disinfection material, their application sequence, as well as their interaction with other bonding agents in assessing shear bond strength.

CHAPTER 3. Conclusions

An experiment was conducted to assess bond strength using a 3-step disinfection technique compared to a conventional bonding technique without using disinfection. Statistical analysis indicated that the 3-step disinfection procedure had significantly higher shear bond strengths ($P<.0001$).

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Appendix A

3-step disinfection group data (MPa)

19,5
22,3
20,8
24,8
29
18
32,5
22
32,5
28,5
24
20
21
19,1
35,8
29,4
25,6
18,8
33
28,8
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27,8
25,3
28
26,2
22,1
27,2
23
24,8
25

Appendix B

Control group data (MPa)

32
27,3
18,5
18,8
20,4
18,8
17,5
23,5
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19,5
17,7
22,5
25,5
18
20,8
17,2
20,5
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19,5
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17,5
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22,1
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22,4
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